Cost Reimbursement Contract

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Terminal Report

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HISTAMINE RELEASE FROM LUNGS OF TEXTILE WORKERS EXPOSED TO COTTON DUST

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Summary of Activities

The activities include the following:

1. Analysis for histamine and histamine releasing activity of cotton dust extracts and derivatives supplied by the SRRC scientists, between October 1979 and March 1981.
2. Histamine challenges - normal laboratory personnel and asthmatic patients have been processed for histamine tests (aerosol inhalation).
3. A textile operative, complaining with respiratory symptoms at work, has been exposed to controlled dust cloud for spirometry and MIAA response.
4. Alternative methods of analysis using liquid chromatography have been pursued in order to identify procedures which quantify histamine release in vivo with greater efficiency than the gas-chromatography method currently used by this laboratory.
5. Extensive research of the literature has been conducted for the purpose of reviewing the published experience on analytical procedures for quantitative measurements of histamine metabolism in vivo.
6. Exchange of information and conferences have been obtained with personnel at the SRRC for the purpose of applying alternative analytical procedures for histamine and metabolites.

2. Human Subject Exposures to Dust

Human subject exposures to either "cotton" dust or cellulose dust have been carried out, using our facilities located in the Clinical Research Unit of North Carolina Memorial Hospital in Chapel Hill. We have also conducted a series of experiments using histamine aerosol inhalation, in order to model the ventilatory response produced by dust clouds. This technique has proven quite suitable to study airways response and histamine metabolites eliminated by exposed subjects (urine).

The results obtained on each subject by gas-chromatography analysis of the MIAA (methyl-imidazole acetic acid in urine) are reported in Table 34.

Monitoring dust exposure with spirometry:

a. Normal Subjects. Healthy volunteers exposed for 1 hr occasionally respond with temporary deflection (loss) of some or all spirometry indicators (see diagrams 1, 2 and 3).

These same subjects, when exposed to "cellulose" dust (Whatman CC-41 cotton derived cellulose) at similar concentrations (1.2-1.5 $\mu g/m^3$) for equivalent duration (1 hr), do not respond.

b. Asthmatic Subjects. Two volunteers with asthmatic complaints, exposed to cotton dust, did not always react to this challenge (diagram 4), suggesting that either the diagnostic label of asthma oversimplifies the issue of airways hyperactivity or that factors other than mere dust concentration are important in these responses.

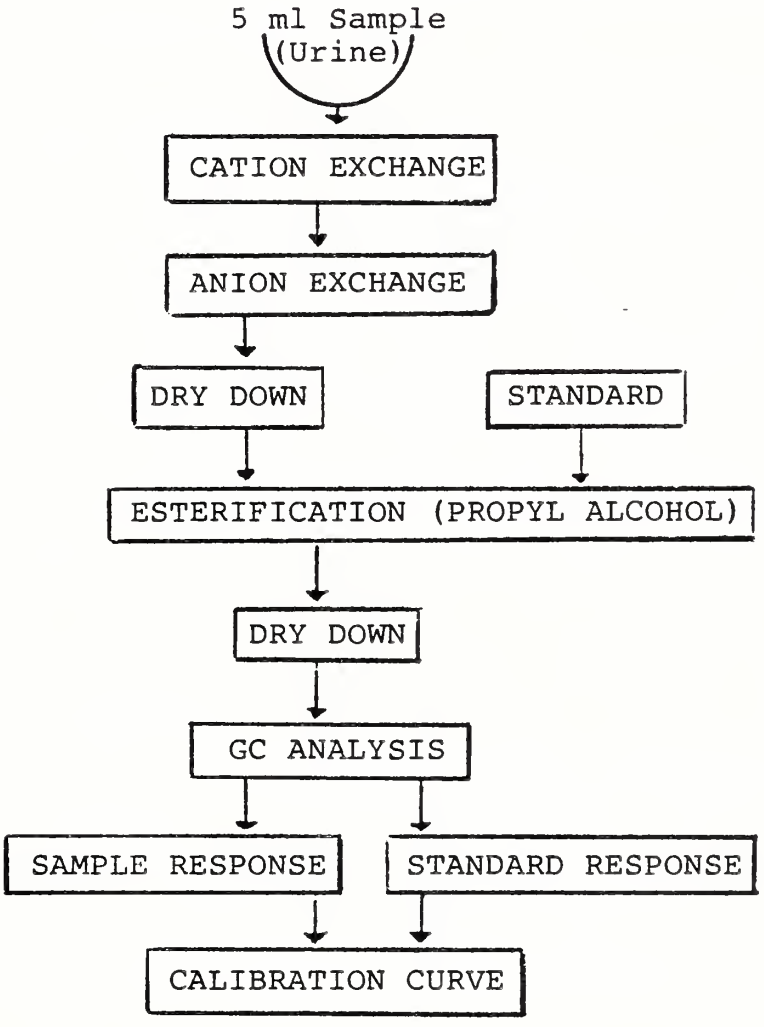
3. Controlled Exposure to Histamine Aerosols

Both normal subjects, as well as asthmatic individuals, display responses of lesser magnitude on the second day of two identical daily exposures experienced in sequence (i.e., within 24 hours).

MIAA ANALYSIS

Method of Tham-McCormick-Zumwalt

(1981)



This phenomenon of tachyphylaxis offers an interesting analogy for the Monday cycle experienced by certain textile workers exposed to cotton dust at the plant.

At any rate, the histamine challenge provides an excellent model to reproduce a ventilatory response analogous to that provoked by dust exposure in "reactive" individuals.

4. Histamine in Urine Samples

The analysis of the histamine and its metabolites in urine samples of exposed subjects, pursued with the high performance liquid chromatography method, has not provided encouraging results. The converging results obtained through several months of investigative activities offer no practical approach for the use of this alternative technique in the detection and quantification of histamine and methyl histamine species in human urine.

5. MIAA and Dust Exposure

The urinary output of MIAA appears to offer an informative indicator of exposure to cotton dust, in that it increased in a textile worker exposed to a controlled cloud of "cotton master blend" dust (dust specimen obtained from the laboratory of North Carolina State University).

6. MIAA and Histamine Exposure

The inhalation of histamine is clearly reflected in an increase of MIAA excretion through the kidneys, paralleling the effect recorded by spirometry immediately following the inhalation.

7. Correlation of MIAA and Exposure Challenge

The limited correlation between spirometry and MIAA post-exposure in dust challenge suggests that in some individuals, MIAA may be a more sensitive indicator of dust inhalation than spirometry.

8. MIAA, Tachyphylaxis and Others

The phenomenon of reduction in MIAA in urine, consistently found in individuals exposed to cellulose dust, is totally unexplained, although it appears to distinguish the response to cellulose from that to cotton dust inhalation.

Publications Related to and Supported by this Grant

1. Battigelli, M.C., Berni, R.J., Sasser, P.E. and Symons, M.J. The Relationship of Acute Respiratory Response and Chronic Respiratory Symptoms in Byssinosis, CHEST, 79S:86-90, 1981.

2. Madden, M.C. and Battigelli, M.C. Bronchial Correlates of Bronchospastic Response to Histamine, Poster Sessions, 2nd Annual Meeting, North Carolina Society of Toxicology, Governor's Inn, January, 1982.

3. Battigelli, M.C., Madden, M.C. and Thomson, M.D. Tachyphylaxis in Histamine Inhalation: Experiments with Normal and Asthmatic Volunteers, Am. Rev. Resp. Dis., 125:254, 1982 (abstract).

4. Battigelli, M.C. Cotton Dust Exposure and Chronic Respiratory Impairment: An Epidemiological Controversy, in Cotton Dust: Controlling an Occupational Health Hazard, J.G. Montalvo, Jr., editor, ACS Symposium Series 189, Am. Chem. Society, Washington, D.C., 1982, pp. 203-212.

5. Battigelli, M.C., Madden, M.C. and Steinsberger, K. Histamine Metabolite in Urine of Subjects Exposed to Histamine and Cotton Dust, Proceedings, Beltwide Cotton Research Conference, S. Antonio, Jan. 4, 1983 (in press).

6. Battigelli, M.C. and Pratt, P. "Toxicology of Cotton Dust", in Progress in Pulmonary Toxicology, G.E.R. Hook, editor, Elsevier Biomedical Press, 1983 (in preparation).

7. Battigelli, M.C. "Does Byssinosis Cause Chronic Airway Disease?", in Current Topics in Occupational Disease, J.B. Gee, editor, Churchill Livingstone, Inc., New York, 1983 (in press).

8. National Research Council, Byssinosis, Clinical and Research Issues, National Academy Press, Washington, D.C., p. 48, 1982.

TABLE 2

HISTAMINE ANALYSIS BY CHEMICAL ASSAY

Content and Release

(November Samples)

1979

Sample	Content (mcg/g dust)	Release (mcg/g lung)
DFB-III-41F	1.4 ± 0.4	0.7 ± 0.9
DFB-III-45-BW	0.4 ± 0.1	0.7 ± 0.6
DFB-III-45-F	1.7 ± 0.3	0.7 ± 0.4
DFB-III-45-G	17.5 ± 3.4	1.3 ± 1.2
DFB-III-45-H	14.0 ± 7.8	NR
DFB-III-48-B	1.4 ± 0.3	9.9 ± 9.1
DFB-III-48-C	1.5 ± 0.5	4.1 ± 2.1
DFB-III-67-B	0.3 ± 0.1	NR
DFB-III-118-A	2.8 ± 0.5	0.2 ± 0.2
DFB-III-119-A	12.0 ± 3.7	0.3 ± 0.7

TABLE 3

BIOLOGICAL ANALYSIS

HISTAMINE CONTENT AND RELEASE

(Samples of October 1979)

Sample	Content (mcg/g dust)	Release (mcg/g lung)
DFB-III-40C	40.0 ± 27.8	2.5 ± 2.2
DFB-III-41A	0.8 ± 0.6	2.3 ± 1.1
DFB-III-41B	29.2 ± 27.7	NR
DFB-III-116A	8.3 ± 6.6	1.8 ± 2.6
DFB-III-54A	8.2 ± 9.2	4.3 ± 2.1
DFB-III-45A	56.3 ± 70.2	1.0 ± 1.0
DFB-III-45C	44.3 ± 22.2	4.5 ± 1.4
DFB-III-45B	1.1 ± 0.8	2.9 ± 3.0
DFB-III-111A	3.4 ± 1.2	0.2 ± 0.5
DFB-III-112A	16.4 ± 3.2	18.7 ± 11.4
DFB-III-112B	183.0* ± 77.9*	9.5 ± 14.8
DFB-III-115-A	1.0 ± 0.2	3.0 ± 3.4

*Questionable results

TABLE 4

BIOLOGICAL ANALYSIS

HISTAMINE CONTENT AND RELEASE

(Samples of November 1979)

Samples	Content (mcg/g dust)	Release (mcg/g lung)
DFB-III-41	0.7 ± 0.6	0.2 ± 0.2
DFB-III-45BW	0.2 ± 0.2	0.1 ± 0.2
DFB-III-45F	5.6 ± 2.6	0.9 ± 0.8
DFB-III-45G	1.5 ± 0.7	2.2 ± 0.9
DFB-III-45H	0.7 ± 1.1	1.0 ± 1.2
DFB-III-48B	0.7 ± 0.3	5.2 ± 5.2
DFB-III-48C	0.5 ± 0.2	2.2 ± 1.7
DFB-III-67B	NR	5.8 ± 6.7
DFB-III-118A	0.8 ± 0.7	1.2 ± 1.1
DFB-III-119A	1.7 ± 1.3	0.6 ± 1.1

Table 1

(USDA Samples Received 4/1/80)

CHEMICAL ASSAY

Sample	Content ug/gm dust*	Release ug/gm lung*	Response	%
LM-0F	0.2 ± 0.1	0.6 ± 1.0	4/8	50%
LM-1F	0.5 ± .3	1.0 ± 1.8	3/8	37.5%
LM-2F	1.0 ± 1.1	0.1 ± 0.2	1/10	10%
LM-3F	0.6 ± 0.3	0.3 ± 0.4	6/9	66.6%
LM-4F	0.5 ± 0.3	0.3 ± 0.5	3/10	30%
LM-5F	0.6 ± 0.3	0.4 ± 0.9	3/10	30%
LM-6F	0.4 ± 0.4	2.1 ± 1.3	10/10	100%

*Standard deviation in parenthesis

Table 2

(USDA Samples Received 4/1/80)

BIOASSAY

Sample	Content ug/gm dust*	Release ug/gm lung*	Response	%
LM-0F	0.4 ± 0.2	0.3 ± 0.5	3/8	37.5%
LM-1F	0.5 ± 0.6	0.1 ± 0.2	1/8	14.3%
LM-2F	0.6 ± 0.3	0.03± 0.1	1/10	10%
LM-3F	0.5 ± 0.3	0.1 ± 0.2	1/10	10%
LM-4F	0.4 ± 0.3	0.3 ± 0.9	1/10	10%
LM-5F	0.3 ± 0.3	0.2 ± 0.5	1/10	10%
LM-6F	0.5 ± 0.5	0.2 ± 0.3	2/10	20%

*Standard deviation in parenthesis

TABLE I

Chemical Analysis of Histamine Content
and Histamine Releasing Effect (±SD)

3/11/81

Sample	Histamine Content mcg/g dust	Histamine Release mcg/g porcine lung
LM-Content	2.2 (± 2.6)	0.9 (± 1.0)
LM-"1"	0.4 (± 0.4)	0.4 (± 0.9)
LM-5	0.7 (± 0.7)	0.1 (± 0.4)
LM-10	1.4 (± 0.9)	0.8 (± 1.9)

TARLE 2

Biological Analysis of Histamine
Content and Histamine Releasing Effect (±SD)
3/11/81

Sample	Histamine Content mcg/g dust	Histamine Release mcg/g lung
LM-Control	1.5 (± 1.9)	0.2 (± 0.2)
LM-"1"	0.7 (± 0.6)	0.04 (± 0.1)
LM-5	0.6 (± 0.5)	0.1 (± 0.1)
LM-10	0.2 (± 0.6)	0.2 (± 0.3)

TABLE 3

Histamine Content and Release
from Miscellaneous Products

(Chemical Analysis)
3/11/81

	Content mcg/g dust	Release mcg/g lung
BURL-F (Brown's dust)	2.5 (± 0.9)	1.9 (± 3.2)
U-16 (Cotton Cellulose)	0.1 (± 0.2)	0.2 (± 0.6)
48/80	--	9.4

TABLE 4

Histamine Content and Release
from Miscellaneous Products

(Biological Analysis)
3/11/81

	Content mcg/g dust	Release mcg/g lung
BURL-F	0.6 (± 0.5)	1.8 (± 1.5)
U-16	0.2 (± 0.2)	0.2 (± 0.3)
48/80	--	26.8

TABLE 1

Histamine Chemical Assay (O-phthaldehyde Method)
Content and Release (Porcine Lung)
12 81

Sample	Histamine Content/Dust mcg/g Dust	Histamine Release mcg/g Lung
LM - 1A	6.1 ± 6.5*	0.3 ± 0.8
LM - 2A	3.8 ± 1.9	0.4 ± 0.6
LM - 3A	7.5 ± 2.8	0.3 ± 0.5
LM - 4A	5.4 ± 3.7	0.8 ± 1.0
LM - 5A	0.2 ± 0.2	0.1 ± 0.2
LM - 1B	1.0 ± 0.9	0.8 ± 0.9
LM - 2B	0.8 ± 0.4	1.5 ± 1.5
LM - 3B	1.4 ± 1.5	1.4 ± 1.6
LM - 4B	1.2 ± 1.2	1.1 ± 1.0
LM - 5B	0.7 ± 1.3	0.5 ± 0.5

*Wide variability of analysis (individual values: 6.7; 3.9; 19.2; 5.8; 19.0; 24.1).

TABLE 2

Histamine Biological Assay (g.p. ileum)
Content and Release (Porcine Lung)
12 81

Sample	Histamine Content/Dust mcg/g Dust	Histamine Release mcg/g Lung
LM - 1A	0.91 ± 0.20	0.30 ± 0.35
LM - 2A	1.41 ± 0.38	0.29 ± 0.27
LM - 3A	3.01 ± 1.27	0.44 ± 0.46
LM - 4A	0.57 ± 0.66	1.04 ± 0.72
LM - 5A	0.08 ± 0.20	ND*
LM - 1B	0.89 ± 0.87	0.27 ± 0.53
LM - 2B	0.20 ± 0.21	0.14 ± 0.30
LM - 3B	0.42 ± 0.61	0.29 ± 0.44
LM - 4B	0.20 ± 0.20	0.08 ± 0.20
LM - 5B	0.19 ± 0.29	0.03 ± 0.08

*ND + less than minimum detectable

TABLE 12

EXPOSURE SESSIONS, OCTOBER 1 - DECEMBER 31, 1982

DATE	TYPE	SUBJECT	CONDITIONS OF EXPOSURE
10/4	Cellulose Dust	MB & MM	1 hr; concentration: 1.53±0.32 mg/m^3
10/5	Cellulose Dust	MB & MM	1 hr; concentration: 1.50±0.27 mg/m^3
10/11	Cotton Master Blend	MB & LA	1 hr; concentration: 1.31±0.32 mg/m^3
10/18	Histamine	MB & MM	1.65% saline aerosol; 10 - 30 breaths
10/19	Histamine	MB & MM	1.65% saline aerosol; 10 - 30 breaths
10/25	Histamine	MB & MM	1.65% saline aerosol; 10 - 30 breaths
10/26	Histamine	MB & MM	1.65% saline aerosol; 10 - 30 breaths
12/16	Histamine	MB	1.65% saline aerosol; 10 - 30 breaths
12/17	Histamine	MB	1.65% saline aerosol; 10 - 30 breaths
12/19	Histamine	MB	1.65% saline aerosol; 10 - 30 breaths
12/20	Histamine	MB	1.65% saline aerosol; 10 - 30 breaths

TABLE 13

EXPOSURE SESSIONS APRIL 8 - SEPTEMBER 14, 1982

DATE	TYPE	SUBJECT	CONDITION OF EXPOSURE
4/8	Histamine	MB	1.65%; 30 breaths; AM; Indocin
4/8	Histamine	MB	1.65%; 30 breaths; PM; Indocin
4/9	Histamine	MB	1.65%; 30 breaths; AM; Indocin
5/14	Master Blend Cotton Dust	MB	30 min; conc: 2.50±1.18
5/20	Master Blend Cotton Dust	MB & EW	1 hr; conc: 1.23±0.42
7/15	Master Blend Cotton Dust	MB & EW	1 hr; conc: 1.48±.50
8/6	Master Blend Cotton Dust	MB & MM	1 hr; conc: 1.37±.44
8/30	Master Blend Cotton Dust	MB & FH	51 min; conc: 1.09±.34
8/31	Master Blend Cotton Dust	MB & FH	51 min; conc: 1.30±.57
9/14	Cellulose Dust	MM & MB	1 hr; conc: 1.26±.34

TABLE 14

EXPOSURE SESSIONS JANUARY 1 - APRIL 7, 1982

DATE	TYPE	SUBJECT	CONDITIONS OF EXPOSURE
1/19	Histamine	EW	0.165%; 30 breaths total
1/20	Histamine	EW	0.165%; 10 breaths
1/21	Histamine	EW	0.165%; 30 breaths
2/3	Histamine	EW	0.165%; 5 breaths
2/4	Histamine	EW	0.165; 5 breaths
2/10	Histamine	MM	1.65%; 15 breaths
2/11	Histamine	MM	1.65%; 15 breaths
2/12	Histamine	MM	1.65%; 15 breaths
2/15	Histamine	FH	0.165%; 5 breaths
2/16	Histamine	FH	0.165%; 5 breaths
2/18	Histamine	FH	0.165%; 5 breaths
2/19	Histamine	FH	0.165%; 5 breaths
4/7	Histamine	MB	1.65%; 30 breaths; A.M.
4/7	Histamine	MB	1.65%; 30 breaths; P.M.

TABLE 15

CELLULOSE EXPOSURE (10 4 82)

		Pre				Post						
		1	2	3	$\bar{X}\pm SD$	1	2	3	4	$\bar{X}\pm SD$	% Δ	t Stat
	VC	4.63	4.73	4.61	4.66±.06	4.59	4.62	4.67	4.61	4.62±.03	.9	.92
	FEV_1	3.63	3.61	3.72	3.65±.06	3.40	3.62	3.36	3.70	3.52±.17	3.6	1.31
MCB	Peak	11.80	11.80	11.56	11.72±.14	11.20	11.48	11.72	12.12	11.63±.39	.8	.37
	25-75	3.95	3.87	4.11	3.98±.12	3.91	4.05	3.97	4.13	4.02±.10	-1.0	--
	50	5.00	5.00	5.20	5.07±.12	4.08	5.24	5.00	5.20	4.88±.54	3.7	.57
	VC	5.64	5.65	5.72	5.67±.04	5.67	5.80	5.73		5.73±.07	-1.1	--
	FEV_1	5.15	5.22	5.45	5.27±.16	4.94	5.31	5.31		5.19±.21	1.5	.57
MCM	Peak	13.28	12.96	12.96	13.07±.18	12.84	12.00	12.84		12.56±.48	3.9	1.69
	25-75	6.76	6.76	6.90	6.81±.08	6.71	6.95	6.93		6.86±.13	-.7	--
	50	7.56	7.88	8.24	7.89±.34	8.00	8.28	8.04		8.11±.15	-2.8	--

* = stat sig α = .05 (t test for means)

** = stat sig α = .01 (t test for means)

TABLE 16

CELLULOSE EXPOSURE (10 5 82)

		Pre				Post					
		1	2	3	$\bar{X}$±SD	1	2	3	$\bar{X}$±SD	% Δ	t Stat
	VC	4.55	4.56	4.71	4.61±.09	4.49	4.59	4.62	4.57±.07	.9	.62
	FEV_1	3.59	3.61	3.62	3.61±.02	3.59	3.66	3.52	3.59±.07	.6	.40
MCB	Peak	11.52	11.24	11.48	11.41±.15	11.36	12.12	11.44	11.64±.42	-2.0	--
	25-75	3.77	3.86	4.04	3.89±.14	3.90	3.86	3.93	3.90±.04	-.3	--
	50	4.96	5.12	5.24	5.11±.14	4.88	5.00	5.16	5.01±.14	2.0	.81
	VC	5.77	5.72	5.80	5.76±.04	5.69	5.54	5.60	5.61±.08	2.6	3.10*
	FEV_1	5.02	5.18	5.13	5.11±.08	5.13	5.00	4.86	5.00±.14	2.2	1.24
MCM	Peak	13.08	13.08	13.08	13.08±0	12.92	12.68	13.32	12.97±.32	.8	.57
	25-75	6.71	7.08	6.71	6.83±.21	6.98	6.97	6.74	6.90±.14	-1.0	--
	50	7.36	8.20	7.52	7.69±.45	8.08	7.48	7.48	7.68±.35	.1	.04

*stat sig $p < .05$

TABLE 17

COTTON DUST CMB EXPOSURE (10 11 82)

Textile worker with respiratory compl.

	Pre				Post						
	1	2	3	$\bar{X}$±SD	1	2	3	4	$\bar{X}$±SD	Δ %	t Stat
VC	2.85	2.82	2.85	2.84±.02	2.63	2.72	2.47	2.84	2.67±.16	-6	1.89
FEV_1	2.26	2.28	2.34	2.29±.04	2.21	2.17	1.95	2.21	2.14±.12	-19	2.07
Peak	5.80	6.92	7.92	6.88±1.06	7.68	7.72	6.96	8.56	7.73±.65	12	-1.32
25-75	2.47	2.59	2.62	2.56±.08	2.53	2.16	1.86	1.94	2.12±.30	-17	2.41
50	4.60	3.56	3.72	3.96±.56	3.64	3.24	2.84	2.76	3.12±.41	-21	2.32

TABLE 18

HISTAMINE INHALATION

(12 16 82)

		Pre				Post					
		1	2	3	X±SD	1	2	3	X±SD	% Δ	t Stat
	VC	4.30	4.38	4.44	4.37±.07	4.15	4.15	4.15	4.14±.01	-5.3	5.60**
MCB	FEV_1	3.42	3.45	3.47	3.45±.03	3.11	2.68	2.92	2.90±.22	-15.9	4.34*
30 br	Peak	11.92	11.80	11.60	11.77±.16	10.00	8.84	8.84	9.23±.67	-21.6	6.40**
1.65%	25-75	4.06	4.00	3.89	3.98±.09	2.74	2.32	2.18	2.41±.29	-39.4	8.95**
	50	5.16	5.00	4.92	5.03±.12	4.00	3.20	3.00	3.40±.53	-32.4	5.19**
	VC	5.75	5.80	5.69	5.75±.06	5.61	5.55	5.65	5.60±.05	-2.6	3.33**
MCM	FEV_1	5.27	5.28	5.09	5.21±.11	4.75	4.79	4.80	4.78±.03	-8.3	6.81**
10 br	Peak	13.64	13.64	12.60	13.29±.60	12.72	12.08	12.64	12.48±.35	-6.1	2.03
1.65%	25-75	6.52	6.22	6.33	6.36±.15	5.53	5.69	5.96	5.73±.22	-9.9	4.12*
	50	7.60	7.08	7.20	7.29±.27	6.20	6.40	6.80	6.47±.31	-11.2	3.50*

* sig stat at $p < .05$

** sig stat at $p < .01$

TABLE 19

HISTAMINE EXPOSURE

(12 17 82)

		Pre				Post					
		1	2	3	X±SD	1	2	3	X±SD	% Δ	t Stat
	VC	4.31	4.40	4.40	4.37±.05	4.19	4.20	4.18	4.19±0.1	-4.1	5.89**
	FEV_1	3.48	3.55	3.51	3.51±.04	3.19	2.56	2.83	2.86±.32	-18.5	3.56*
MCB	Peak	11.04	11.24	11.12	11.13±.10	10.12	9.00	8.60	9.24±.79	-17.0	4.13*
	25-75	3.80	3.84	3.98	3.87±.09	3.04	2.37	2.23	2.55±.42	-34.1	5.19**
	50	4.88	5.04	5.12	5.01±.12	4.20	3.20	3.00	3.47±.64	-30.7	4.09*
	VC	5.62	5.51	5.61	5.58±.06	5.50	5.44	5.74	5.56±.16	-.4	.20
	FEV_1	5.20	4.59	5.29	5.03±.38	4.72	4.46	4.74	4.64±.16	-7.8	1.63
MCM	Peak	13.40	12.56	13.64	13.20±.57	11.72	11.48	12.12	11.77±.32	-10.8	3.79*
	25-75	6.52	6.33	6.57	6.47±.13	5.34	5.23	5.02	5.20±.16	-19.6	10.73**
	50	7.64	6.92	7.44	7.33±.37	6.08	5.80	5.52	5.80±.28	-20.9	5.71**

* sig stat at $p < .05$

** sig stat at $p < .01$

TABLE 20

HISTAMINE EXPOSURE
(10 18 82)

		Pre				Post					
		1	2	3	$\bar{X}\pm SD$	1	2	3	$\bar{X}\pm SD$	% Δ	t Stat
	VC	5.51	5.51	5.60	5.54±.05	4.90	5.40	5.36	5.22±.28	-5.8	1.96
MCM	FEV_1	4.85	4.27	4.79	4.64±.32	4.62	4.11	4.85	4.53±.38	-2.4	.38
10 br	Peak	12.72	12.40	12.88	12.67±.24	12.24	12.04	11.80	12.03±.22	-5.1	3.37*
1.65%	25-75	6.56	6.75	6.78	6.70±.12	6.37	5.70	5.95	6.01±.34	-10.3	3.33*
	50	7.60	8.00	8.08	7.89±.36	6.88	6.32	6.64	6.61±.28	-16.2	5.82**
	VC	4.57	4.59	4.54	4.57±.03	4.30	4.32	4.36	4.33±.03	-5.3	10.50**
MCB	FEV_1	3.69	3.62	3.70	3.67±.04	3.15	3.08	3.04	3.09±.06	-15.8	14.21**
30 br	Peak	11.80	12.12	12.16	12.03±.20	9.76	9.00	8.60	9.12±.58	-24.2	8.10**
1.65%	25-75	3.95	3.98	4.06	4.00±.06	2.75	2.16	2.23	2.38±.32	-40.5	8.55**
	50	4.84	4.88	5.00	4.91±.08	3.68	2.84	3.00	3.17±.45	-35.4	7.23**

* sig stat at $p < .05$

** sig stat at $p < .01$

TABLE 21

HISTAMINE EXPOSURE

(10 19 82)

		Pre				Post					
		1	2	3	$\bar{X}\pm SD$	1	2	3	$\bar{X}\pm SD$	% Δ	t Stat
	VC	5.62	5.34	5.67	5.54±.18	5.45	5.35	5.47	5.42±.06	2.2	1.10
MCM	FEV_1	5.16	5.09	5.02	5.09±.07	2.91	4.82	4.91	4.21±1.13	-17.3	1.34
10 br	Peak	13.20	12.96	12.92	13.03±.15	11.08	11.88	12.56	11.84±.74	-9.1	2.72
1.65%	25-75	6.42	7.14	6.60	6.72±.37	5.80	5.68	5.50	5.66±.15	-15.8	4.54*
	50	7.08	8.16	7.80	7.68±.55	6.76	6.32	6.16	6.41±.31	-16.5	3.47*
	VC	4.43	4.55	4.58	4.52±.08	4.31	4.34	4.29	4.31±.03	-4.7	4.30*
MCB	FEV_1	3.50	3.63	3.58	3.57±.07	3.19	2.99	2.98	3.05±.12	-14.6	6.61**
30 br	Peak	10.72	11.40	11.64	11.25±.48	9.92	9.52	9.48	9.64±.24	-14.3	5.22**
1.65%	25-75	3.68	3.95	3.81	3.81±.14	2.52	2.30	2.19	2.34±.17	-38.6	11.87**
	50	4.76	5.08	4.96	4.93±.16	3.48	3.12	2.92	3.17±.28	-35.7	9.33**

* = stat sig at $p < .05$

** = stat sig at $p < .01$

TABLE 22

HISTAMINE EXPOSURE

Analysis of data from Tab 8

	MCB %Δs		MCM	
	Day 1	Day 2	Day 1	Day 2
V_C	-5.3	-4.7 ↓	-5.8	-2.2 ↓
FEV_1	-15.8	-14.6 ↓	-2.4	-17.3 ↑
Peak	-24.2	-14.3 ↓	-5.1	-9.1 ↑
25-75	-40.5	-38.6 ↓	-10.3	-15.8 ↑
50	-35.4	-35.7 -	-16.2	-16.5 -

TABLE 23

HISTAMINE EXPOSURE

(10 25 82)

		Pre				Post					
		1	2	3	$\bar{X}$±SD	1	2	3	$\bar{X}$±SD	% Δ	t Stat
MCM	VC	5.64	5.53	5.64	5.60±.06	5.57	5.32	5.56	5.48±.14	-2.14	1.34
	FEV_1	5.12	4.42	4.14	4.56±.50	5.09	4.64	4.83	4.85±.23	+6.36	-.92
	Peak	13.84	13.64	12.80	13.43±.55	11.92	11.64	11.76	11.78±.20	-12.29	5.03**
	25-75	6.51	7.03	6.62	6.72±.27	6.11	5.91	5.74	5.92±.19	-11.90	4.19*
	50	7.64	8.28	7.88	7.93±.32	6.80	6.44	6.44	6.56±.21	-17.28	6.19**
MCB	VC	4.42	4.56	4.64	4.54±.11	4.31	4.37	4.39	4.36±.04	-3.97	2.67
	FEV_1	3.23	3.64	3.73	3.53±.27	3.11	3.02	3.04	3.06±.05	-13.3	3.05*
	Peak	11.20	11.52	11.80	11.51±.30	9.72	8.96	8.88	9.19±.46	-20.16	7.28**
	25-75	3.85	3.61	3.85	3.77±.14	2.74	2.18	2.09	2.34±.35	-37.93	6.56**
	50	5.00	4.92	4.92	4.95±.05	4.00	3.08	2.84	3.31±.61	-33.13	4.63**

*stat sig $p < .05$

**stat sig $p < .01$

TABLE 24

HISTAMINE EXPOSURE
(10 26 82)

		Pre				Post					
		1	2	3	$\bar{X}\pm SD$	1	2	3	$\bar{X}\pm SD$	% Δ	t Stat
MCM	VC	5.56	5.77	5.81	5.71±.13	5.58	5.59	5.81	5.66±.13	-.88	.49
	FEV_1	4.89	5.21	5.21	5.10±.18	4.49	4.85	4.99	4.78±.26	-6.27	1.78
	Peak	12.80	13.16	13.36	13.11±.28	11.72	12.16	12.60	12.16±.44	-7.25	3.13*
	25-75	6.44	6.59	6.47	6.50±.08	5.46	5.65	5.63	5.58±.10	-14.15	12.15**
	50	7.00	7.24	7.24	7.16±.14	6.04	6.44	6.32	6.27±.21	-12.43	6.25**
MCB	VC	4.38	4.55	4.58	4.50±.11	4.34	4.43	4.36	4.38±.05	-2.67	1.86
	FEV_1	3.24	3.43	3.60	3.42±.18	3.23	2.88	3.03	3.05±.18	-10.82	2.59
	Peak	11.12	11.12	11.32	11.19±.12	10.40	9.80	9.20	9.80±.60	-12.42	3.93*
	25-75	3.95	3.86	3.88	3.90±.05	2.82	2.23	2.24	2.43±.34	-37.69	7.45**
	50	4.96	4.96	4.84	4.92±.07	3.84	3.00	3.08	3.31±.46	-32.72	5.96**

*stat sig $p < .05$

**stat sig $p < .01$

TABLE 25

Analysis of data from Table 24

	MCB %Δs		MCM	
	Day 1	Day 2	Day 1	Day 2
VC	-3.97	-2.67 ↓	-2.14	-.88 ↓
FEV_1	-13.3	-10.82 ↓	+6.36	-6.27 ↑
Peak	-20.16	-12.42 ↓	-12.29	-7.25 ↓
25-75	-37.93	-37.69 -	-11.90	-14.15 ↑
50	-33.13	-32.72 -	-17.28	-12.43 ↓

TABLE 26

Histamine inhaled in scalar doses

12/19/82	FEV_1s		12/20/82	FEV_1s	
Pre	3.36		Pre	3.50	
	3.51			3.55	
	3.37			3.52	
	3.41±.08			3.52±.03	
		(% Δ from baseline X̄)			(% Δ from baseline)
10 br	3.12	(8.5%)	10 br	3.01	(13.1%)
	3.16	(7.3%)		3.10	(11.9%)
	2.96	(13.2%)		3.08	(13.1%)
	3.08±.11	(9.7%↓)		3.06±.05	(13.1%↓)
20 br	2.59	(24.1%)	20 br	2.96	(15.9%)
	2.71	(20.5%		2.83	(19.6%)
	2.92	(14.4%)		2.78	(21.0%)
	2.74±.17	(19.6%↓)		2.86±.09	(18.6%↓)
30 br	2.38	(30.2%)	30 br	2.78	(21.0%)
	2.64	(22.6%)		2.94	(16.5%)
	2.81	(17.6%)		2.64	(25.0%)
	2.61±.22	(23.5%↓)		2.79±.15	(20.7%↓

Statistics: Significance * $p < .05$, ** $p < .01$, t-test for means

	Base	10 br	20 br	30 br
Baseline	--	4.28*	6.24**	5.99**
10 br	4.28	--	3.13*	3.38*
20 br	6.24**	3.13*	--	0.82
30 br	5.99**	3.38*	0.82	--

	Base	10 br	20 br	30 br
Baseline	--	14.88**	12.00**	8.38**
10 br	14.88**	--	3.43*	3.04*
20 br	12.00**	3.43*	--	.69
30 br	8.38**	3.04*	.69	--

t-test for means NS between baselines (2.18); 10 breaths (1.65); 20 breaths (0.25); and 30 breaths (0.60). ( ) - t stat value.

TABLE 27

Histamine, scalar doses, $FEF_{50\%}$

12/19/82		12/20/82	
Pre	3.87 3.84 3.95 3.89±.06	Pre	4.00 4.00 3.75 3.92±.14
10 br	2.92 2.58 2.53 2.68±.21 (↓31.1%)	10 br	2.93 2.43 2.41 2.59±.29 (↓33.9%)
20 br	2.36 2.12 2.11 2.20±.14 (↓43.4%)	20 br	2.40 2.09 2.08 2.19±.18 (↓44.1%)
30 br	2.32 2.08 2.12 2.17±.13 (↓44.2%)	30 br	2.20 2.15 2.05 2.13±.08 (↓45.7%)

TABLE 28

Histamine, scalar doses, FEF_{25-75}
Changes from baseline in brackets

12/19/82		12/20/82	
Pre	4.96 4.60 4.96 4.84±.21	Pre	5.40 5.16 4.88 5.15±.26
10 br	4.28 3.68 3.52 3.83±.40 (+20.9%)	10 br	4.20 3.40 3.52 3.71±.43 (+28.0%)
20 br	2.92 2.84 2.72 2.83±.10 (+41.5%)	20 br	3.40 2.84 2.80 3.01±.34 (+41.6%)
30 br	2.84 2.60 2.80 2.75±.13 (+43.2%)	30 br	3.08 3.08 2.92 3.03±.09 (+41.2%)

TABLE 29

Histamine, scalar doses, $FIF_{50\%}$

Changes from baseline in brackets

12/19/82		12/20/82	
Pre	5.37 6.21 5.81 5.80±.42	Pre	6.87 6.47 6.63 6.66±.20
10 br	6.87 6.84 6.45 6.72±.23 (↑15.9%)	10 br	6.56 5.97 5.55 6.03±.51 (↓9.5%)
20 br	5.81 5.97 5.97 5.92±.09 (↑2.1%)	20 br	6.44 5.97 6.90 6.44±.47 (↓3.3%)
30 br	5.39 6.29 4.70 5.46±.80 (↑6.2%)	30 br	6.39 6.18 6.55 6.37±.19 (↓4.4%)

TABLE 30

Histamine scalar doses, $FIF_{50\%}$

Changes from baseline in brackets

12/9/82		12/20/82	
Pre	4.88 5.84 5.48 5.40±.48	Pre	6.60 6.28 6.44 6.44±.16
10 br	6.28 6.28 6.04 6.20±.14 (↑14.8%)	10 br	6.32 5.97 5.36 5.88±.49 (↓8.7%)
20 br	5.44 5.16 5.68 5.43±.26 (↓0.6%)	20 br	6.32 5.60 6.60 6.17±.52 (↓4.2%)
30 br	5.04 5.80 4.36 5.07±.72 (↓6.1%)	30 br	5.88 5.76 6.08 5.91±.16 (↓8.2%)

TABLE 31

Histamine, scalar exposures, FIVC

Subject:MB

1/10/82		1/11/82	
Pre	8.00 7.08 6.76 7.28±.64	Pre	6.60 6.48 4.08 5.72±1.42
10 br	6.32 5.80 5.76 5.96±.31 (↓18.1%)	10 br	6.80 6.72 6.88 6.80±.08 (↑18.9%)
20 br	5.64 4.60 5.76 5.33±.64 (↓26.8%)	20 br	6.84 6.24 5.76 6.28±.54 (↑9.8%)
30 br	5.96 5.68 5.80 5.81±.14 (↓20.2%)	30 br	5.96 5.88 4.96 5.60±.56 (↓2.1%)

TABLE 32

Histamine, scalar doses, FIVC

MB

12/19/82		12/20/82	
Pre	4.10	Pre	4.18
	4.26		4.23
	4.26		4.30
	4.21±.09		4.24±.06
10 br	4.26	10 br	4.17
	4.27		3.89
	4.19		4.17
	4.24±.04		4.08±.16
20 br	4.07	20 br	4.14
	4.22		4.22
	4.23		4.20
	4.17±.09		4.19±.04
30 br	4.06	30 br	3.98
	4.27		4.26
	4.15		4.32
	4.16±.11		4.19±.18

TABLE 33

Histamine, scalar doses, Peak Flow

Changes from baseline in brackets

MB

12/19/82			12/20/82		
Pre	11.04		Pre	11.44	
	11.32			11.40	
	11.64			11.64	
	11.33±.30			11.49±.13	
		(% Δ from Pre X̄)			(% Δ from Pre X̄)
10 br	10.68	(5.7)	10 br	10.44	(9.1)
	10.08	(11.0)		9.92	(13.7)
	9.44	(16.7)		9.60	(16.4)
	10.07±.62	(↓11.1%)		9.99±.42	(13.1%)
20 br	8.28	(26.9)	20 br	9.00	(21.7)
	8.20	(27.6)		9.20	(19.9)
	7.80	(31.2)		8.84	(23.1)
	8.09±.26	(↓28.6%)		9.01±.18	(21.6%)
30 br	7.68	(32.2)	30 br	8.80	(23.4)
	8.04	(29.0)		9.04	(21.3)
	8.20	(27.6)		8.72	(24.1)
	7.97±.27	(30.0%)		8.85±.17	(23.0%)

Between days

Pre	.85
10 br	.51
20 br	4.31*
30 br	4.17*

*stat sig $p < .05$

TABLE 34

MIAA

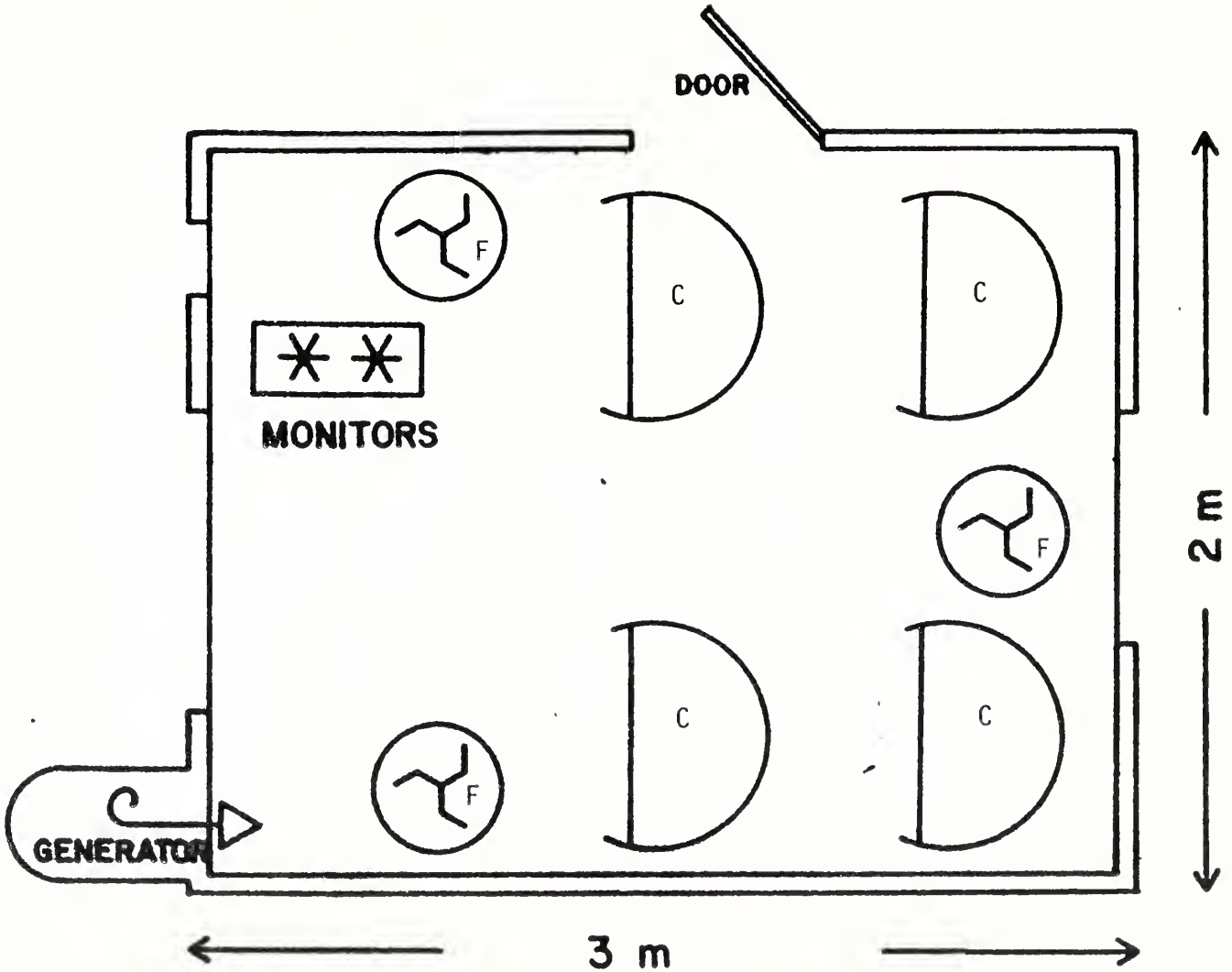
URINARY OUTPUT, 2 hr, BEFORE AND AFTER EXPOSURE

DATE	TYPE	SUBJECT	BEFORE MIAA µg/hr±SD	AFTER MIAA sig/hr±SD	DIFFERENCE	P
10/4	Cellulose	MB	57±2	35±2	-39	> 0.05
10/5	Cellulose	MB	52±3	45±9	-14	> 0.05
10/4	Cellulose	MM	63±4	54±5	-14	> 0.05
10/5	Cellulose	MM	61±2	24±5	-61	> 0.05
10/18	Histamine	MB	70±8	153±14	119	< 0.01
10/19	Histamine	MB	67±3	106±21	58	> 0.05
10/25	Histamine	MB	83±5	157±5	89	< 0.01
10/26	Histamine	MB	113±5	169±5	50	< 0.01
10/18	Histamine	MM	73±12	156±9	114	< 0.01
10/19	Histamine	MM	109±3	135±0	24	< 0.01
10/25	Histamine	MM	79±3	96±18	22	> 0.05
10/26	Histamine	MM	93±9	88±14	-5	> 0.05
10/11	Cotton Dust	LA	123±10	156±3	27	< 0.01

Diagram 1

EXPOSURE ROOM

UNC - CH

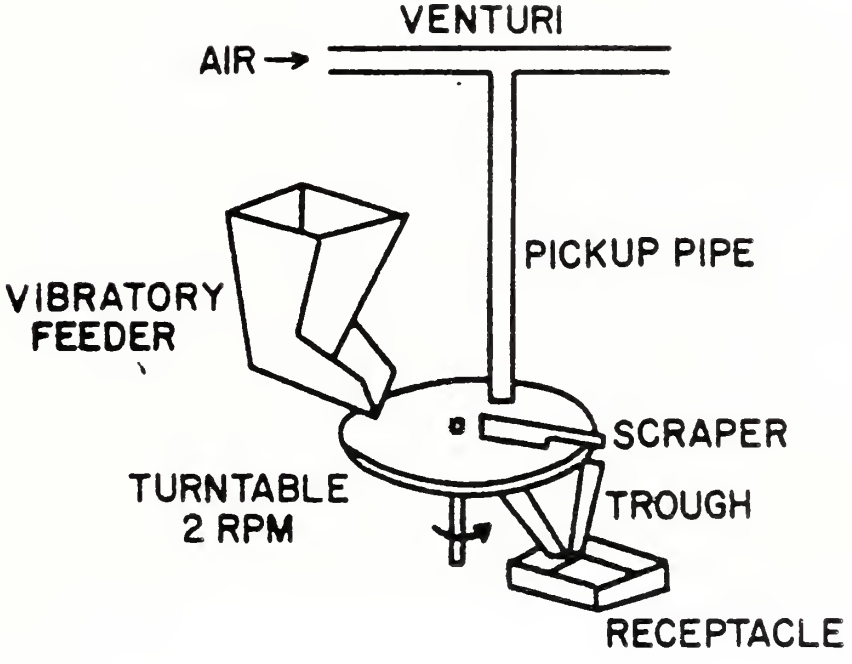


C = Chair

F = Fan

Diagram 2

Dust injecting apparatus



HARVARD TURNTABLE FEEDER-AEROSOL GENERATOR

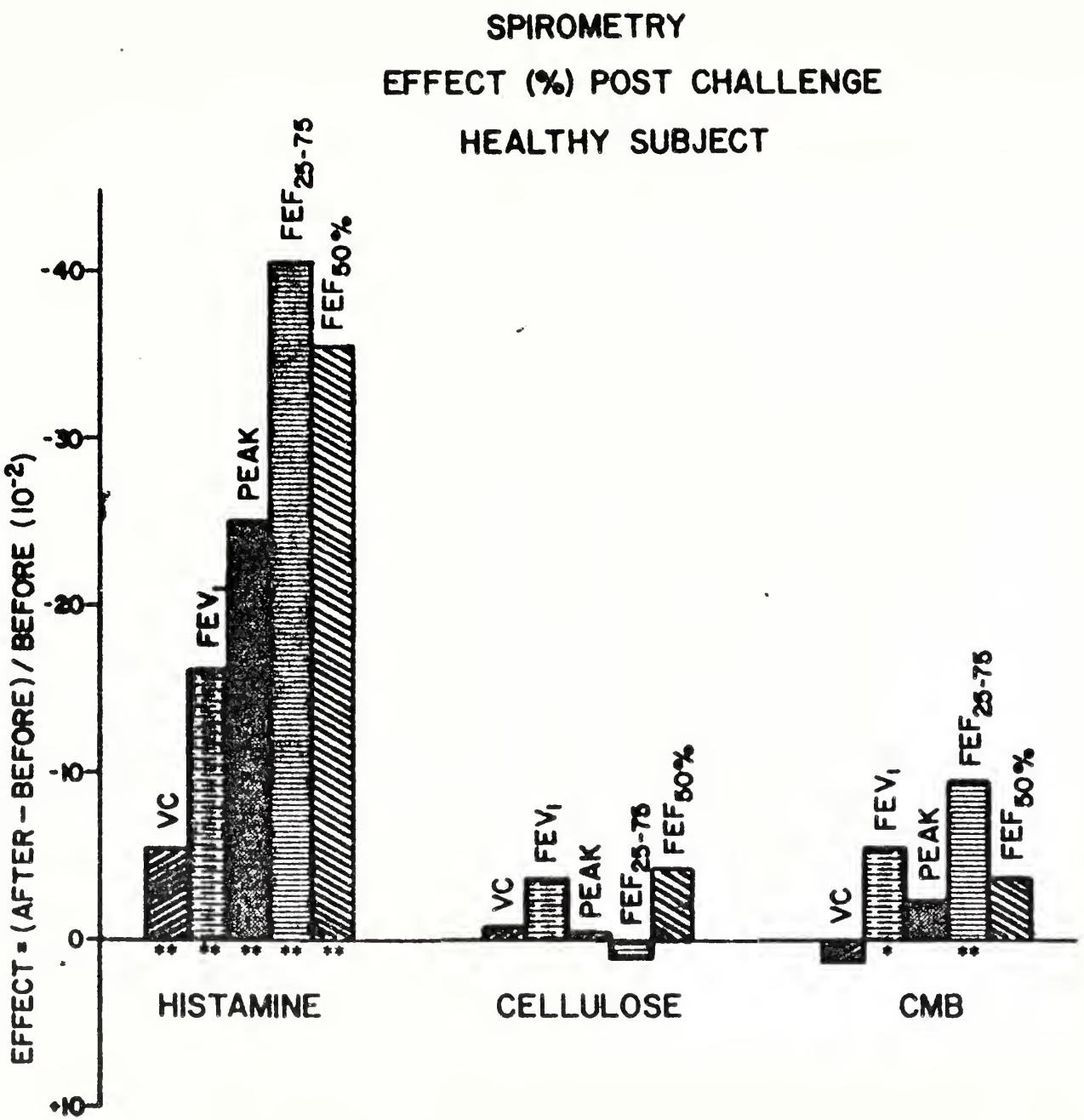
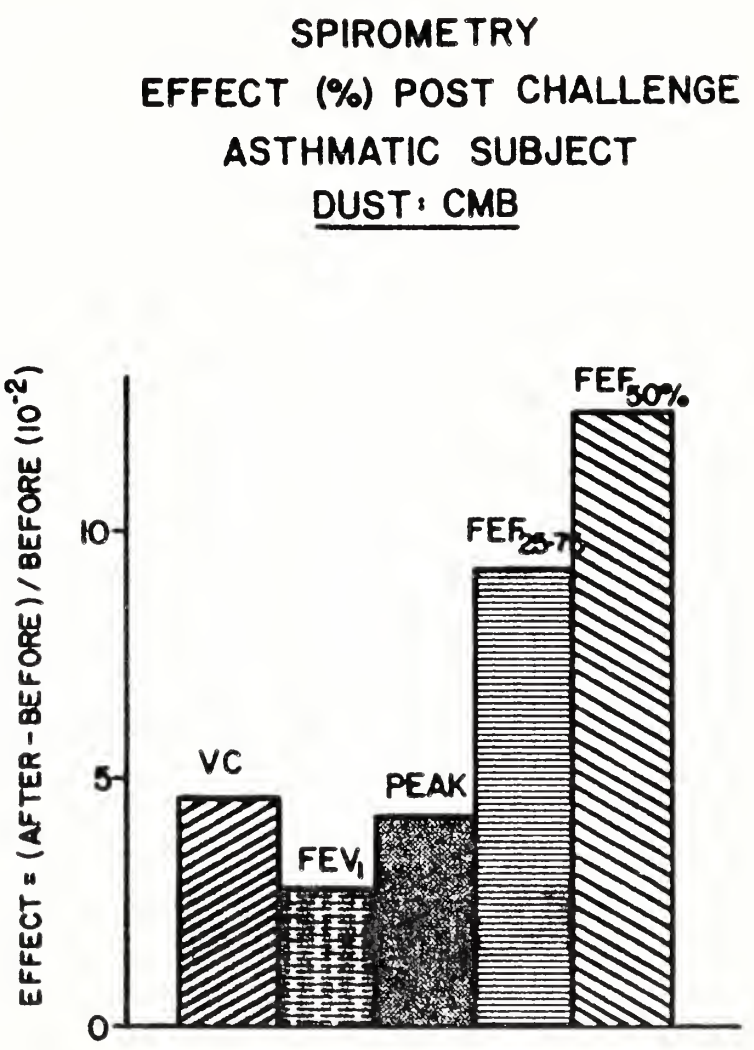


Diagram 3 : Ventilatory response to different challenges, normal subject.

Diagram 4 : Ventilatory response of "sensitive" subject (asthmatic) to cotton dust.



APPENDIX A

QUANTITATION OF URINARY HISTAMINE AND METHYLHISTAMINE: A SUMMARY OF HPLC METHODOLOGY EXPLORED

by Michael C. Madden

CONTENTS

I. Introduction

In view of the technical difficulties connected with the analysis of histamine and methylhistamine in urine by the spectrophotometry, a systematic approach has been devoted to the exploration of the high performance liquid chromatographic (HPLC) method. It should be stated that at the time this research was initiated, an extensive review of the published literature failed to disclose a rapid and reproducible method for this application (i.e., histamine and methylhistamine analysis). Several derivatives have been tried, as well as different sequences, in terms of chromatographic separation following or preceding the derivatization step.

II. Precolumn Derivatization of Histamine

1. o-phthaldialdehyde

Davis, et. al. (1978) published a method for analysis of histamine in urine tissue, and plasma utilizing the reaction between histamine and o-phthaldialdehyde (OPT or OPA) before injecting the resulting highly fluorescent product into a high performance liquid chromatograph (HPLC). We attempted to use the Davis method for determination of histamine concentrations in urine.

The lowest detection limit we could achieve with the apparatus employed (Model 6000 pump, UK6 Injector, 10 μm μBondapak phenyl stainless steel column, Model 420C Fluorometer, and a Model 730 Data Module; all Waters Associates make) was 2 ng in a standard solution (Fig. 1), while Davis, et. al. (1978) reported their lowest detection limits as less than

100 pg in a standard solution. The reason for the discrepancy in lower detectable limits may be that the 420 Fluorometer has a 10 µl flow cell, while the other group uses a Schoeffel Spectrofluoro Monitor with a 5 µl flow cell. The excitement and emission wavelengths (340 nm and 425 nm long pass, respectively) were comparable in both fluorometers.

Our lower detection limits for the histamine fluorophor were found to be linear (Fig. 2), which is necessary for working with low levels of urinary histamine.

Davis, et. al. (1978) reported the stability of the histamine-OPT fluorophor to be 100 hours when stored under N_2 at 4°C in ethyl acetate. Our fluorophor broke down within one day when stored under those conditions at either 4°C or -60°C. Furthermore, during the processing of urine samples, the fluorophor may decay before the analysis is completed. Tables 1 and 2 show the decay of the histamine and the ^{1,4}methylhistamine fluorophors, documented by their peak heights decreasing. The histamine fluorophor's half-life is approximately 66 minutes, while the methylhistamine fluorophor's is even shorter (26.5 minutes). Our findings that the histamine fluorophor decays quite rapidly is not surprising; Gardner and Miller (1980) found similar results with eighteen amino acids reacted with OPT.

Other disadvantages associated with this method were low flow rates (generally less than 2.0 ml/min.), long chromatogram run times (30 min. or more), and rapid degeneration of the expensive stainless steel column due to deterioration of the packing by urinary contaminants, even with a guard column preceding the injection into the analytical apparatus.

2. Fluorescamine

Fluorescamine (Fluram®, Roche Diagnostics) was proposed as a reagent to complex with primary amines, resulting in a fluorophor (Weigele, et. al.,

1972). We attempted to utilize this chemical for histamine analysis.

The pH for optimum reactivity and formation of the fluorophor was pH 9.0 (Table 3). This pH was used in all subsequent derivatizations with fluorescamine. The column used in HPLC work was a radial compression cartridge, C_{18} packing, 10 μm particle size, in a water compression module. However, authentic urine samples showed no detectable levels of either histamine or methylhistamine (Fig. 3). The fraction where histamine elutes from the column is masked by a large interfering peak which could not be eliminated by a Bio Rex 70 cleanup (discussed in Section IV).

3. Dansyl Chloride

Dansyl chloride forms a very stable product when reacted with histamine under basic conditions (Table 4). The complex's peak shape was vastly improved when chromatogrammed on a 5 μm C_{18} particle size column packing, as opposed to the 10 μm particle size (Fig. 4).

The method of Seiler, et. al. (1978) was followed using a C_{18} packing. The results of various determinations of urinary histamine with this method showed erratic percent recoveries, as well as non-detectable amounts of the amine in urine (Table 5).

III. Post Column Derivatization of Histamine

1. OPT

Urinary histamine was reacted with OPT after it had been separated from other urinary compounds on a cyano (CN) cartridge, 10 μm packing. The coupling was allowed to occur in a nine foot stainless steel coil, 0.03" diameter at 55°C (see Wall, et. al., 1982).

The retention of histamine on the CN column was influenced by the solvent's acidity, amount injected, and the presence or absence of sodium lauryl sulfate in the solvent (Tables 6, 7, 8 and 9). Histamine stability, a standard solution stored at -20°C, was assessed to be excellent (Table 10).

Urinary histamine concentrations ranged between 6.0 mg to 37.1 mg/24 hours as determined by this HPLC method (Table 11). These values are a thousand fold higher than other literature values, e.g., 42 µg/24 hr for 15 subjects (range from 21-65 µg/24 hr), determined by fluorescence (Oates, et. al., 1962) and 16±14 µg/24 hr for 16 individuals, determined by a radioisotope assay (Beaven, et. al., 1972). Possibly, there is an interfering compound which elutes with the same retention time as histamine, resulting in higher than normal values with the HPLC method.

IV. Urine Clean Up Methods

1. Bio-Rex 70 Resin

Lewis and Fennessy (1981) used a weak cation exchange resin, Bio-Rex 70 (BioRad Laboratories), for column chromatography in order to clean up blood and tissue extracts before analyzing for histamine fluorometrically. This idea was also utilized by Oates, et. al. (1962) with another weak cation exchange resin, IRC-50 (Fisher Scientific Co.). We have utilized the same principle for urine clean up before derivatization with either OPT or fluorescamine and subsequent injection into the HPLC.

Our results show that there are interfering compounds in the buffer solutions used in the column-chromagraphy procedure which interfere with the detection of the urinary histamine - fluorescamine fluorophor

(Figs. 5, 6 and 7). Similar results were obtained with the histamine - OPT fluorophor. Alterations of the run conditions, i.e., flow rates, percent organic solvent, and solvent pH, did not substantially improve the chromatograms.

2. Sep-Pak

A C_{18} Sep-Pak cartridge (Waters Assoc.) was used to clean up an OPT derivatized sample of urine. Subden, et. al. (1978) had used the same method for analysis of histamine in urines. No significant improvement resulted in the urine's chromatogram.

A Sep-Pak clean up of urine before OPT derivatizing was attempted with a C_{18} cartridge, but this did not remove interferences.

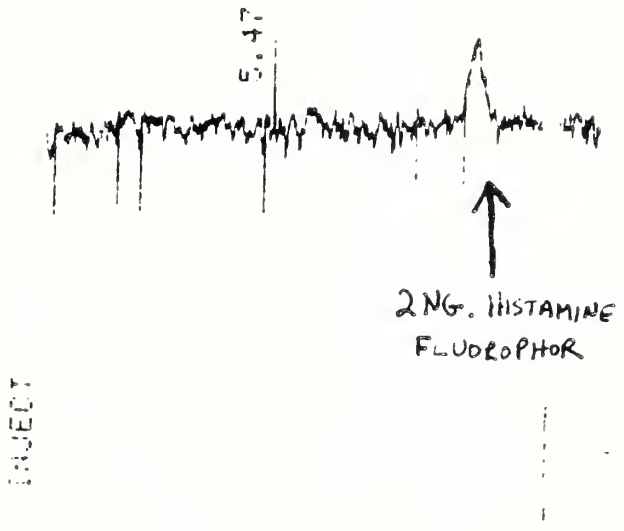


Fig. 1. 2 ng of histamine-OPT fluoraphor; this is the lowest detection limit for histamine on our system using the method of Davis, et. al.

Run conditions:

45% MeOH-PO_4 buffer solvent, 1.5 ml/min. μ Bondapak phenyl column, 10 μm packing.

HISTAMINE FLUOROPHOR DETECTION

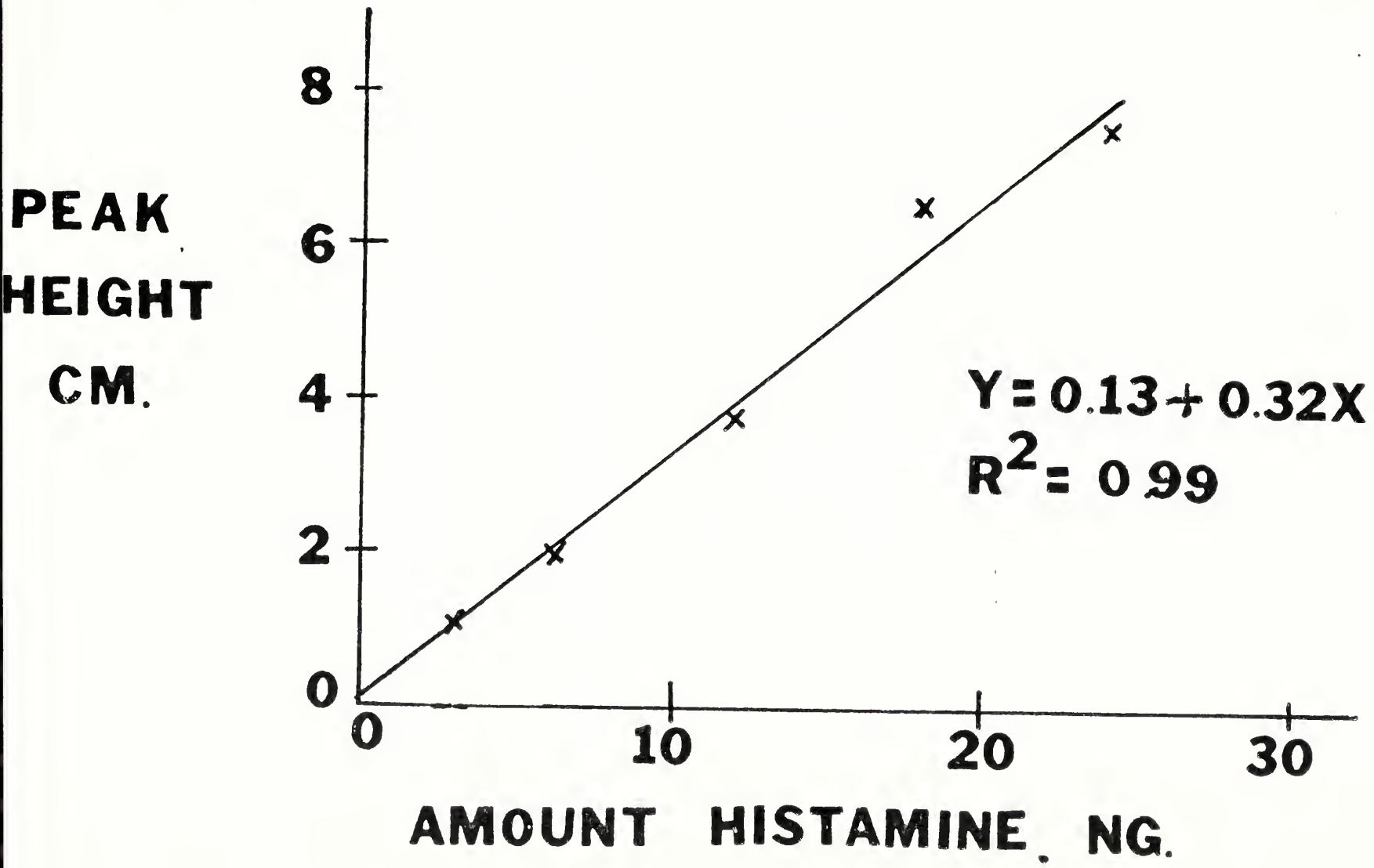


Fig. 2. Linear detection range of histamine-OPT fluorophor. Method according to Davis, et. al. (1978).

TABLE 1. DECAY OF HISTAMINE-OPT FLUOROPHOR WITH TIME

Time After Derivatization (min)	Volume Injected (µl)	Peak Area ($x10^6$) (relative units)
2	1.0	120.8
8	1.0	111.9
16	1.0	108.1
24	1.0	106.5
33	1.0	103.2
39	1.0	89.5
46	1.0	102.2
53	1.0	78.5
60	1.0	82.5
66	1.0	60.6
109	1.0	45.0
173	1.0	54.7

Assay Conditions:

0.5 ml histamine solution added to 0.5 ml OPT solution and vortex 5 sec.; allow to react for 55 sec. more before adding 1.0 ml HCl. Inject sample after 60 more sec.

Histamine solution: 100 µg/ml

OPT solution: Dissolve 1.6 gm OPT in 30 ml ethanol. Add to a boric acid solution (24.6 gm in 1 l water) and adujst pH to 10.4. Add 1.0 ml meracapto Ethanol.

TABLE 2. DECAY OF METHYLHISTAMINE-OPT FLUOROPHOR WITH TIME

Time After Derivatization (min)	Volume Injected (µl)	Peak Height x10^3 (relative units)
2.0	50	121.0
10.0	50	90.4
18.5	50	74.4
26.5	50	59.9
34.0	50	48.0
42.0	50	42.0
50.0	50	40.3
57.5	50	29.5

Assay conditions as in Table 1, but use 100 µg/ml methyl-histamine instead of histamine.

TABLE 3. OPTIMUM pH FOR THE DERIVATIZATION OF HISTAMINE BY FLUORESCAMINE

Vol. Solution Injected (µl)	Retention (min)	pH Borate Buffer	Peak Height rel. units
50	7.30	7.5	105,142
50	7.30	8.0	106,577
50	7.30	8.5	126,286
50	7.30	9.0	130,528
50	7.20	9.5	103,869
50	7.20	10.0	83,439

Assay was to vortex 0.5 ml histamine (100 ng/µl) and 1.0 ml 0.2M sodium borate solution of different pH for 15 sec; 50 µl of the solution was then injected 1 min. after reacting.

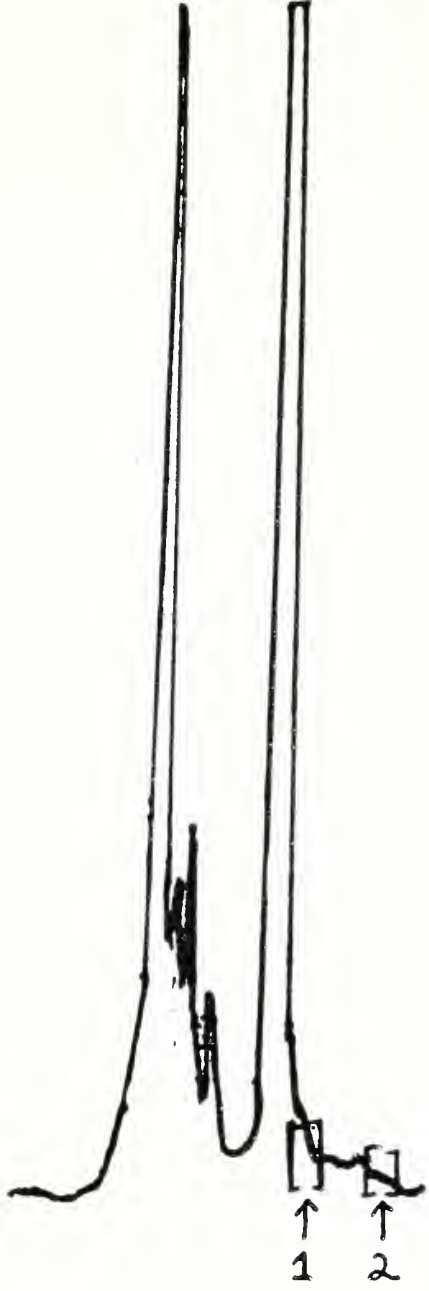


Fig. 3. Urine sample reacted with fluorescamine solution. [] with number 1 shows when histamine should elute; this area is masked by interfering compound. [] number 2 shows no methylhistamine eluting in this region.

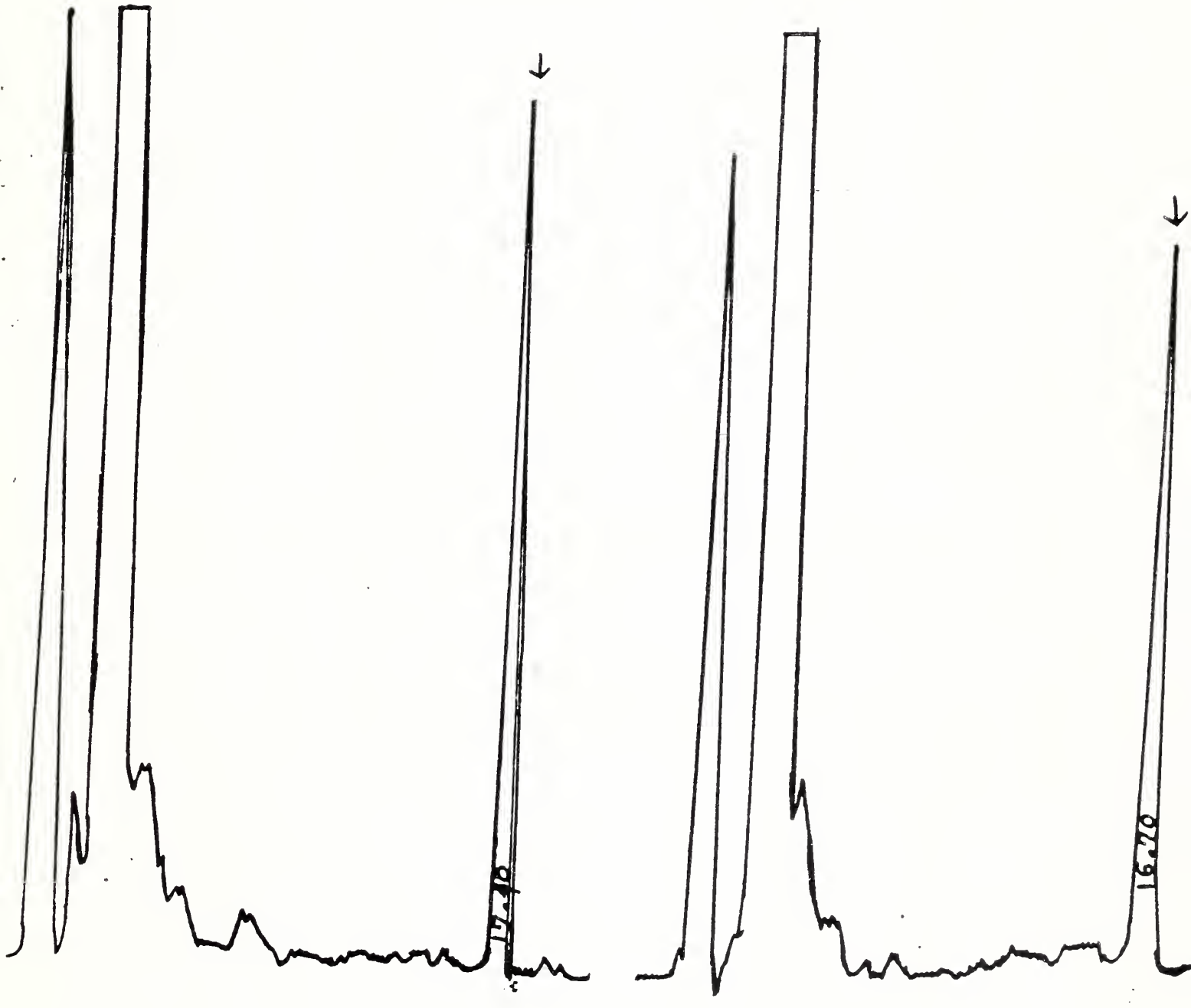
TABLE 4. STABILITY OF THE HISTAMINE - DANSYL CHLORIDE FLUOROPHOR

(All samples were kept on ice.)

Time From First Injection (min)	Volume Injected (µl)	Retention (min)	Peak Height (relative units)
0	50	21.30	72,297
35	50	21.40	65,949
70	50	20.70	70,636
143	50	20.70	67,577
191	50	20.70	68,882
226	50	20.70	68,708
			$\bar{X}\pm SD$ = 69,008±2,235

Figure 4.

The use of finer packing material (#C_{18}) appears to improve the spike of authentic histamine dissolved in urine.



5 um C_{18} packing

10 um C_{18} packing

TABLE 5.

Summary of Dansyl-derivative Analysis

Urine	[Histamine]	Spiked Urine % Recoveries	Volume Urine used for Reaction (ml)
MCM (unchallenged)	ND		3.0
MCM (unchallenged)	616 ug/l		3.0
EAW unchallenged	ND		3.0
EAW (post cotton dust)	ND	176, 426	4.0
EAW (post cotton dust)	ND	534	4.0
EAW (post cotton dust)	ND	1248, 1112	4.0
MCM (post cotton dust)	$\frac{362\ ug}{845\ ml}$	97.5, 64.5	6.0
MCM (post cotton dust)	$\frac{19\ ug}{845\ ml}$	93.5, 104.5 110.0, 99.1 (101.8±7.1)	5.0
MCM	ND		5.0
MCB (unchallenged)	ND	10.0, 6.6, 68.4 (28.3±34.7%)	5.0 (reacted at 46°C for 1 hr)
MCB (unchallenged)	ND		5.0 (reacted at 46°C for 1 hr)
MCB (post cellulose exposure)	ND		5.0

ND=not detected

TABLE 6

INFLUENCE OF pH ON RETENTION TIME
Method According to Wall, et. al. (1982). OPT

pH 25% CH_3CN (2 ml/min)	Time (min) Histamine	Methylhistamine	Integration Availability of Emerging Peak
2.30	--	@ 9.3	no
2.04	3.42	@ 5.0	no
1.81	--	@ 3.0	no
1.71	1.50	@ 1.58	yes

Histamine reaction was accomplished in 9' of 0.03" ID tubing at 54°C. HLC column used was a 10 µm radical compression cyano (CN) cartridge.

TABLE 7

RETENTION TIME VS. SIZE OF INJECTION

Histamine Injected (ng)	Retention Time (min) ± Standard Deviation (n = 4 Injections)
10	5.35 ± .05
50	5.01 ± .00
100	4.91 ± .05
200	4.71 ± .03

Assay conditions as in Table 6. Solvent pH was 2.30.

TABLE 8. EFFECT OF SODIUM LAURYL SULFATE ON HISTAMINE RETENTION TIME

Run	Histamine Injected (ng)	Retention Time (min)
1	500	11
2	100	14
3	100	17
4	100	21

Assay: 0.02% sodium lauryl sulfate (SLS) was added to the HPLC solvent (25% CH_3CN, pH 2.00 $\bar{w}$ H_2SO_4). Column: cyano RCM cartridge, 10 µm; flow rate 1.0 ml/min. Post column derivatization $\bar{w}$ OPT.

TABLE 9. EFFECT OF REMOVAL OF SODIUM LAURYL SULFATE FROM SOLVENT ON THE RETENTION TIME OF HISTAMINE

Run	Histamine Injected (ng)	Retention Time (min)
1	100	13.89
2	100	10.37
3	100	9.27
4	100	7.84
5	100	6.51
6	100	5.50
7	100	5.52
8	100	5.00

Same system as in Table 8. Run 1 was done $\bar{w}$ SLS in the solvent; the solvent was then replaced with the CH_3CN lacking SLS for runs 2-8.

TABLE 10

TEST OF STABILITY AT -20°C
OF A HISTAMINE SOLUTION IN WATER

Days of Storage	Number of Tests	Conc. (ng/µl)	% Recovered by HPLC
1	4	10	104.3
	4	100	104.3
2	4	10	95.2
	4	100	110.1
3	4	10	104.4
	4	100	95.5
6	4	10	97.9
	4	100	100.7

TABLE 11

TOTAL HISTAMINE EXCRETION IN 24 HOURS
AS DETERMINED BY THE WALL, ET. AL. (1978) METHOD

Day	Subject	Histamine Concentration ng/µl	Total Excreted 24 hrs (mg.)	
1	o→, 26 yrs.	5.06	8.3	
1	o→, 55 yrs.	10.81	16.1	Histamine Challenge
2	o→, 55 yrs.	4.95	6.0	
3	o→, 55 yrs.	27.57	30.0	
4	o→, 55 yrs.	27.28	37.1	

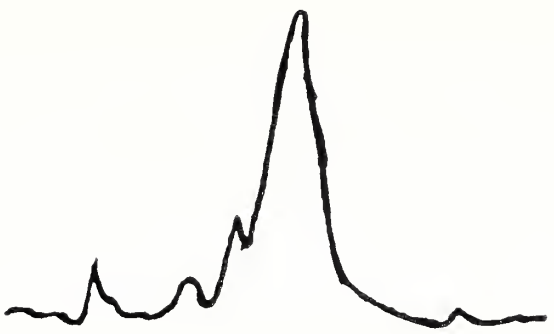


Figure 5. Chromatogram of blank run through Bio Rex 70 resin and eluted with HCl. Blank consisted of 20 ml water and 10 ml Na_2HP_{04} buffer at pH 7.5.

Figure 6. Chromatogram of urine run through Bio Rex 70 resin. No histamine is detectable since the Contaminant Peak trails over where histamine would elute ([ ]).

Figure 7. Same sample as run in Figure 6, but spiked with histamine. The arrow indicates this peak.

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APPENDIX B

HISTAMINE METABOLITE IN URINE OF SUBJECTS EXPOSED TO HISTAMINE AND COTTON DUST

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Abstract

The controlled exposure of normal and asthmatic subjects to histamine and card-room cotton dust causes a measurable increase in the urinary output of methylimidazole acetic acid (MIAA). Two hour collection samples obtained immediately before and following these exposures indicate that MIAA may provide a sensitive and independent indicator of exposure in fasting individuals whether histamine is inhaled or released by these challenges.

Introduction

The physician's pressing concern is understanding the nature of the reaction to the inhaled dust, namely the process leading to the self-limited ventilatory effect of byssinosis.

An understanding of this phenomenon, such as provided by a mediator of response released by the actions of dust deposited on the respiratory surfaces, would undoubtedly assist in the precise definition of the ventilatory response, enabling a more satisfactory diagnostic differentiation between trivial responses and significant injurious effects. Secondly, this clarification would permit an objective monitoring of the effect by a measurement independent of the ventilatory response, or parallel and associated with it, but conveniently available as a marker of the significant exposure. Most important, the objective identification of a biochemical marker would

permit a more precise and quantitative assessment of the mechanism, assisting not only in the environmental control of the textile exposure (i.e., determining the benefits of washing, dust control, etc.), but also in the medical management of workers exposed (i.e., relocation, ventilatory protection, dismissal, etc.).

Mechanism of Action in Byssinosis: The Range of Hypothesis

The airways in mammals are endowed with exquisite plasticity controlled by the bronchial musculature responding to inhaled stimuli through one or more of four major mechanisms: local neuro-reflex effector (irritant receptors) (1); naso-bronchial reflex (2); humoral effector (mediator release) (3); and direct stimulation (mechanical or biochemical) (4).

The quantitative studies of these responses have disclosed that certain individuals react in an exaggerated fashion to stimuli which are not effective in the majority of healthy subjects. This hyperreactivity, or, as some author prefers to call, hyperresponsiveness (5), is particularly enhanced in asthmatic subjects, but it is also present although to a lesser extent, in a variety of other chronic disorders, including chronic bronchitis and emphysema (6,7,8), as well as in cigarette smokers (6). Normal subjects often present some degree of exaggerated response, with a frequency variously given between 3% and 35% (6,7,9). These figures may be vastly exceeded when "normal" subjects, that is subjects free of chronic respiratory disorders, are tested during or following an upper respiratory infection (10).

The airway reactivity of subjects with a history of byssinosis has been investigated yielding results of conflicting meaning. While some authors report presence of hyperresponsive behavior in workers exposed to cotton dust (11,12,13) others do not (14,15).

Biochemical Correlates of Byssinosis: Urinary MIAA

Studies monitoring histamine released by cotton dust in human volunteers were attempted first by Bouhuys, et. al. (16), and later by Edwards, et. al. (17). The conclusions of these researchers suggest a surge of the major metabolite of histamine, the methylimidazole acetic acid (MIAA) in the urine of exposed subjects. The interpretation of these studies, however, is hampered by the limited details given in these papers in regard to the statistical basis and the variability of these observations.

Present Study: Methods

In order to extend and verify the findings of Bouhuys (11) and Edwards (17), a panel of subjects composed of two healthy adult, two asthmatic and one byssinotic subjects were exposed to provocative challenge (aerosolized dust or histamine) in order to study the effects of these exposures on MIAA in urine. These observations require extensive preparatory work to standardize the analytical procedure, the timing of urine collection, and to finalize the protocol of experiment for measuring surge of urinary MIAA following challenge. MIAA is commonly and abundantly present in normal urine, and the assessment of a small increase dependent on the challenge requires particular care.

The volunteer subjects were processed after receiving instruction on the procedure, having signed the consent form, fasted, and been free of medication for the 12 hours preceding the test. The baseline two hour urine collection and the baseline spirometry were obtained immediately before the test. At the conclusion of the exposure (either histamine aerosol or dust, 1.3 mg/m^3 for 1 hr) the "effect" spirometry was obtained. The "effect" urine collection was timed to begin at the outset of each exposure, and was completed two hours later. Replicas were obtained for each of the exposure modalities (dust or histamine). A few exposure sessions to dust were obtained with inert particulate clouds, using cotton derived cellulose of respirable size (Whatman cc41).

The analytical method employed for the determination of MIAA is a gas chromatographic procedure based on that published by Tham, et. al. (18) and modified by McCormick, et. al. (19), with the introduction of a double ion exchange column preceding the GC analysis of the propylester.

Results

Monitoring MIAA in urine, with collections of two hour samples in fasting subjects, allows a reproducible and informative index of histamine metabolism. Although MIAA output tends to vary from day to day in the same subject, the monitoring of this metabolite during consecutive intervals in the same day in the fasting individual shows a rather uniform range of values (see Table 1).

The exposure to histamine aerosol (0.16-1.6% buffered solution) influences the immediate excretion of MIAA in urine, with a measurable

increase ranging from 50% up to 119% of baseline values, several folds higher than observed in consecutive samples obtained free of challenge (i.e., spontaneous variability, see Table 2). Noteworthy is the trend indicating a loss of increment in the output of the second consecutive day, following an identical inhalation (i.e., same dose in two consecutive days). The exposure to cellulose dust, a challenge which has little influence, if any, on spirometry, does not cause an increase, but rather a neglibible reduction of the metabolite (see Table 3).

With cardroom dust, normal subjects present a conspicuous increment of MIAA output, even when these subjects fail to respond with a deflection of spirometry (Table 4). With asthmatic individuals, the increase in MIAA follow a similar trend, reaching peak values higher than the amounts excreted by normal subjects (Table 5). The only byssinotic subject exposed to similar dustiness does react with a MIAA increase comparable to, but not as high as those displayed by brittle asthmatic subjects.

Conclusions

In summary, the following conclusions appear warranted:

1. Normal subjects challenged with histamine via aerosol increase their output of MIAA immediately following the experimental inhalation.
2. Repeating this histamine challenge in consecutive days, a reduced MIAA output is noted on the second day, relative to the first.
3. Exposure to cellulose dust, which is ineffective on spirometry, does not increase the MIAA output, at least in normal subjects.

4. Exposure to cotton dust does increase the MIAA in the urine of both normal and asthmatic subjects, with the latter experiencing the highest relative increments.
5. MIAA monitored in the urine of subjects exposed to cotton dust offers a sensitive, although not a specific, indicator of exposure, with a limited parallel to the spirometric responses, in that the former may increase even though spirometric changes are not noted.

Acknowledgements

We acknowledge the assistance received from Dr. J.P. McCormick, University of Missouri, Columbia, Mo. This research was made possible by support from Cotton, Inc. of Raleigh, N.C. and the US Department of Agriculture, SRRC, New Orleans, LA.

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Table 1. MIAA Output baseline (μg/hr). Sequential two hour urine collections (normal subject). Mean ±50 of three replicates.

Sample	1	2	3	4
	82±11	74±0	84±6	70±8

Table 2. MIAA output following challenge with histamine (normal subject).

Subject	Week	(Day)	Dose° (mg)	Pre (μg/hr)	Post (μg/hr)	% Difference
A	I	(1)	23	70±8	153±14	119**
A	I	(2)	31	67±3	106±21	58
A	II	(1)	15	83±5	157±5	89**
A	II	(2)	18	113±5	169±5	50**

°Estimate based on aliquot aerosolized during inspiratory phase. Amount actually inhaled is not directly measured.

**significant at $p \leq .01$, t-test for means.

Table 3. MIAA urinary output (μg/hr). Cellulose dust exposure (normal subjects)

Subject	Dust ($mg/m^3/hr$)	MIAA (μg) Pre	Post	% Difference
A	1.47±0.17	52±2	56±1	+8 (NS)
A	1.50±0.25	57±5	35±2	-39 (NS)
A	1.53±0.23	52±3	45±9	-14 (**)
B	1.47±0.17	105±4	66±11	-37 (**)
B	1.50±0.27	63±4	54±5	-14 (NS)
B	1.53±0.23	61±2	24±2	-61 (**)

NS=non-significant
**significant at $p \leq 0.01$, t-test for means.

Table 4. MIAA urinary output. Cotton dust exposure, healthy subjects not responding (unchanged spirometry)

Subject	Dust ($mg/m^3/hr$)	MIAA (µg) Pre	MIAA (µg) Post	% Difference
A	1.48	90±15	66±6	-27 (NS)
A	1.44	60±2	100±9	+50 (*)
A	1.29	55±2	112±7	+67 (**)
A	1.51	48±3	82±13	+71 (**)
B	1.44	85±6	115±14	+30 (**)

NS=non-significant
*=significant at $p \leq 0.05$, t-test for means.
**=significant at $p \leq 0.01$

Table 5. MIAA urinary output. Cotton dust exposure, asthma (Ast) and byssinosis (Bys) subjects

Subject	Dust	MIAA (µg) Pre	MIAA (µg) Post	% Difference
C (Ast)	1.23	76±6	190±40	+150 (**)
C (Ast)	1.48	48±0	95±7	+65 (**)
D (Ast)	1.29	96±3	136±14	+34 (**)
D (Ast)	1.51	55±2	150±5	+173 (**)
E (Bys)	1.31	123±10	156±3	+27 (**)

**=significant at $p \leq 0.01$, t-test for means.